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Original Article

Rosiglitazone Induced Smooth Muscle Relaxation and Reversal Via L-Bisphenol A Diglycidyl Ether

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ABSTRACT

Background: PPAR-γ (Peroxisome Proliferator Activated receptor gamma) ligands are capable of suppressing the expression of a variety of genes involved in inflammation present in the macrophages upon activation and carry the potential of being the anti-inflammatory targets for diseases of airways. Agonists of PPAR-γ including thiazolidinediones can, therefore be utilized as a potential treatment for inflammatory diseases of the airways such as asthma and COPD (chronic obstructive pulmonary disease).

Objective: To establish the bronchodilatory effect of Rosiglitazone

Methodology: The study was performed using the isolated pieces of smooth muscle of the trachea of total 20 guinea pigs divided into four groups. Contractions were noted via an oscillograph having four channels with the help of a displacement transducer. Effects of Rosiglitazone on the smooth muscles of the trachea were noted through adding histamine and then blocking the PPAR receptors via addition of BADGE (L-Bisphenol A Diglycidyl Ether).

Results: The group given histamine showed the mean ± standard error of mean of the contractions varying from 10 to 83 mm. A decrease in the histamine induced contractions was noted on addition of Rosiglitazone. BADGE enhanced the histamine induced contractions and reduced the effect of rosiglitazone on histamine induced contractions. The obtained values were utilized for plotting curves of semi log and were compared among different groups.

Conclusion: The results solidify the hypothesis that thiazolidinediones produce the relaxation of the smooth muscles via activation of PPAR gamma receptor.

Key words; PPAR- γ, Thiazolidinediones, NF-kB, Interleukins, COPD.

Introduction

Thiazolidinediones (TZDs) are the drugs that increase the sensitization towards insulin and act to decrease insulin resistance through forming a bond with the gamma receptor of peroxisome proliferator gene (PPAR-γ). [1] There have been a number of studies over the last few years that show the therapeutic benefits of TZDs beyond their use in diabetes such as anti-inflammatory action in diseases including Alzheimer's disease, pancreatitis and inflammatory airway diseases .[2]

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PPAR-γ ligands are capable of suppressing the expression of a variety of genes involved in inflammation present in the macrophages upon activation. [3]

Ligands of PPAR-γ including TZDs are involved in the proliferation of cells and carcinomas. [4] The proliferator activated receptors of peroxisome (PPARs) are one of the types of hormonal nuclear receptors. [5] High expression of PPAR-α is seen in tissues which exhibit high rates of catabolism of fatty acids e.g. hepatic, cardiac, renal as well as intestinal tissues. Expression in the epithelium of the lung is quite high .[6]

Both the, α and γ receptors have immune-modulating properties and hence carry the potential of being the anti-inflammatory targets for diseases of airways .[7] PPARs also act via the repression of gene transcription of pathways involving inflammation e.g. nuclear factor-kB (NF-kB). Hence the therapeutic potential of the ligands to these receptors in asthmatic conditions

have been postulated. [8] TZDs show a significant reduction in the inflammation of the lung and production of mucus along with a decrease in the production of interferons, IL-4 and IL-2 by the T type cells .[9] TZDs regulate the epithelial inflammatory response markedly. Agonists of PPAR-γ receptors can therefore be utilized as a potential treatment for inflammatory diseases of the airways such as asthma and COPD (chronic obstructive pulmonary disease).

BADGE (Bisphenol A diglycidyl ether) antagonizes the γ receptor of PPAR.[10, 11] Since rosiglitazone brings about its effect on the respiratory smooth muscles through action of the PPAR gamma, therefore by using BADGE, the role of PPAR-γ can be exploited in the effects of TZDs on tracheal smooth muscle.

In this study we observed the effects of Rosiglitazone on the smooth muscles of the trachea through adding histamine and then blocking the PPAR receptors via addition of BADGE.

Methodology

The study was performed using the isolated pieces of smooth muscle of the trachea on total 20 guinea pigs (male as well as female) belonging to the class of Dunkin-Hartley [12] variety with body weight ranging from 500-600g. [9] The sample size was calculated via following formula;

Sample size = Degree of freedom / No. of groups + 1

Animals were divided into four groups. They were placed at animal house of Army Medical College, and maintained at optimal temperature, food and water. Statistics were calculated as percentages through Microsoft excel.

1. **Construction of Concentration-Response-Curve of Histamine (10^{-7} to 10^{-3} M)**

After equilibration was achieved, a concentration of 10^{-7} M of histamine was poured in the organ bath. The recording was done via an oscillograph having four channels with the help of a displacement transducer. A total of 6 tests were done following the same procedure. The data obtained from the tests was utilized to plot the concentration curves of the response to histamine. Washing off of the drug was done after the achievement of a plateau effect.

After the restoration of the tension on the baseline, the steps of the experiment were repeated using histamine in concentrations of 10^{-6}, 10^{-5} M, 10^{-4} M as well as 10^{-3} M. A total of 6 tests were done following the same procedure. The data obtained from the tests was utilized to plot the concentration curves of the response to histamine.

2. **Construction of Concentration-Response-Curve of Histamine in Fixed Rosiglitazone Concentration (100 μM)**

After equilibration was achieved, a concentration of 10^{-7} M of histamine was poured in the organ bath in a fixed rosiglitazone concentration of 100 μM. The recording was done via an oscillograph having four channels with the help of a displacement transducer. A total of 6 tests were done following the same procedure. The data obtained from the tests was utilized to plot the concentration curves of the response to histamine in rosiglitazone. The data obtained from the tests was utilized to plot the concentration curves of the response to histamine. Washing off of the drugs was done after the achievement of a plateau effect.

After the restoration of the tension on the baseline, the steps of the experiment were repeated using histamine in concentrations of 10^{-6}, 10^{-5} M, 10^{-4} M as well as 10^{-3} M in fixed rosiglitazone concentration. A total of 6 tests were done following the same procedure. The data obtained from the tests was utilized to plot the concentration curves.

3. **Construction of Concentration-Response-Curve of Histamine in Fixed BADGE Concentration (100 μM)**

After equilibration was achieved, a concentration of 10^{-7} M of histamine was poured in the organ bath in a fixed BADGE concentration of 100 μM. The recording was done via an oscillograph with four channels with the help of a displacement transducer. A total of 6 tests were done following the same procedure. The data obtained from the tests was utilized to plot the concentration curves of the response to histamine in BADGE. The data obtained from the tests was utilized to plot the concentration curves of the response to histamine. Washing off of the drugs was done after the achievement of a plateau effect.

After the restoration of the tension on the baseline, the steps of the experiment were repeated using histamine in concentrations of 10^{-6}, 10^{-5} M, 10^{-4} M as well as 10^{-3} M in fixed BADGE concentration. A total of 6 tests were done following the same procedure. The data obtained from the tests was utilized to plot the concentration curves.

4. **Construction of Concentration-Response-Curve of Histamine in Fixed Rosiglitazone (100 μM)and BADGE Concentration (100 μM)**

After equilibration was achieved, a concentration of 10^{-7} M of histamine was poured in the organ bath in a fixed Rosiglitazone and BADGE concentration of 100 μM. The recording was done via an oscillograph

having four channels with the help of a displacement transducer. A total of 6 tests were done following the same procedure. The data obtained from the tests was utilized to plot the concentration curves of the response to histamine in Rosiglitazone and BADGE. The data obtained from the tests was utilized to plot the concentration curves of the response to histamine. Washing off of the drugs was done after the achievement of a plateau effect.

After the restoration of the tension on the baseline, the steps of the experiment were repeated using histamine in concentrations of 10^{-6}, 10^{-5} M, 10^{-4} M as well as 10^{-3} M in fixed Rosiglitazone and BADGE concentration. A total of 6 tests were done following the same procedure. The data obtained from the tests was utilized to plot the concentration curves.

Results

Group I - Histamine Group

The group given histamine showed the mean ± standard error of mean of the contractions varying from 10 to14, 24 to 34, 47 to 52, 60 to 72 and 75 to 83 mm (Table 1). The 10-3 M was taken as 100% and based on that the other percentages was calculated to be 14.29, 39.02, 63.54 and 83.58 percent (Table 1). The obtained values were utilized for plotting curves of semi log (Fig 1).

Table 1: Group 1 (Smooth muscle response to histamine)

Concentration (M) of Histamine	Amplitude of Contraction (mm ± S.E.M) n = 10	Percent (%) Response
10^{-7}	11.17 ± 0.65	14.29
10^{-6}	30.5 ± 1.63	39.02
10^{-5}	49.67 ± 0.71	63.54
10^{-4}	65.33 ± 1.98	83.58
10^{-3}	78.17 ± 1.30	100

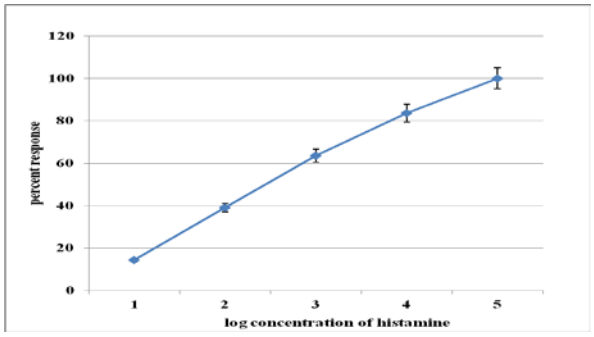

Figure 1: Log concentration response curve of histamine on isolated tracheal muscle of guinea pig.

Group 2

Histamine & Rosiglitazone Group:

There was a significant change noticed in the contractions induced by histamine, when rosiglitazone was added (Fig 2). This group showed mean ± SEM amplitudes varying from 7.17 ± 0.31 at 10^{-7}, 23.3 ± 1.17 at 10^{-6}, 42.5 ± 0.96 at 10^{-5}, 55.3 ± 0.56 at 10^{-4} and 69.2 ± 0.91 mm at 10^{-3}. The resultant contractility was noticed in the range from 6 to 8, 20 to 27, 39 to 45, 53 to 57 and 66 to72 (Table 2). The percentages of the observed responses were calculated as 9.17, 29.85, 54.37, 70.79 and 88.49 percent. The obtained values were utilized for plotting curves of semi log (Fig 2).

Table 2: Group 2 (Response of histamine and Rosiglitazone

Concentration (M) of Histamine	Amplitude of Contraction (mm ± S.E.M) n = 10	Percent (%) Response
10^{-7}	7.17 ± 0.31	9.17
10^{-6}	23.33 ± 1.17	29.85
10^{-5}	42.5 ± 0.96	54.37
10^{-4}	55.33 ± 0.56	70.79
10^{-3}	69.17 ± 0.91	88.49

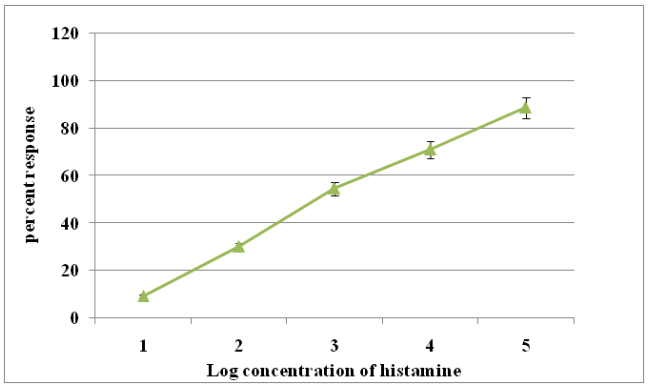

Figure 2: Curve of histamine and Rosiglitazone

Group 3- BADGE Group

This group showed mean ± SEM amplitudes varying from 10.67 ± 0.67, 31.83 ± 1.67, 50.83 ± 1.01, 65.17 ± 0.95 and 77.83 ± 1.30 mm. The resultant contractility was noticed in the range from 9 to 13, 29 to 37, 48 to 55, 62 to 68 and 74 to 82 mm. The percentages of the observed responses were calculated as 13.65, 40.72, 65.03, 83.37, and 99.57 percent (Table 3). The obtained values were utilized for plotting curves of semi log (Fig 3).

Table 3: Group 3 Effect of BADGE on Histamine Contractions

Concentration (M) of Histamine	Amplitude of Contraction (mm ± S.E.M) n = 10	Percent (%) Response
10^{-7}	10.67 ± 0.67	13.65
10^{-6}	31.83 ± 1.67	40.72
10^{-5}	50.83 ± 1.01	65.03
10^{-4}	65.17 ± 0.95	83.37
10^{-3}	77.83 ± 1.30	99.57

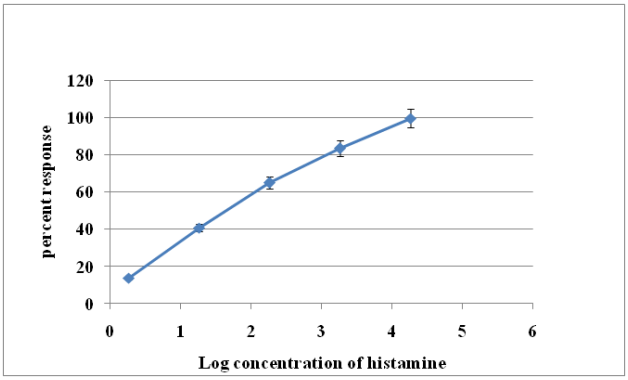

Figure 3: Effect of BADGE on Histamine Contractions

Group 4- Rosiglitazone & BADGE Group

This group showed mean ± SEM amplitudes varying from 17.67 ± 0.56, 23 ± 1.00, 42.5 ± 1.43, 57.67 ± 1.89 and 69.67 ± 1.28 mm. The resultant contractility was noticed in the range from 6 to 10, 20 to 25, 39 to 49, 53 to 64 and 66 to 75mm. The percentages of the observed responses were calculated as 9.81, 29.42, 54.37, 73.78 and 89.13 percent (Table 4). The obtained values were utilized for plotting curves of semi log (Fig 4).

Table 4: Response of rosiglitazone+BADGE administration

Concentration (M) of Histamine	Amplitude of Contraction (mm ± S.E.M) n = 10	Percent (%) Response
10^{-7}	7.67 ± 0.56	9.81
10^{-6}	23 ± 1.00	29.42
10^{-5}	42.5 ± 1.43	54.37
10^{-4}	57.67 ± 1.89	73.77
10^{-3}	69.67 ± 1.28	89.13

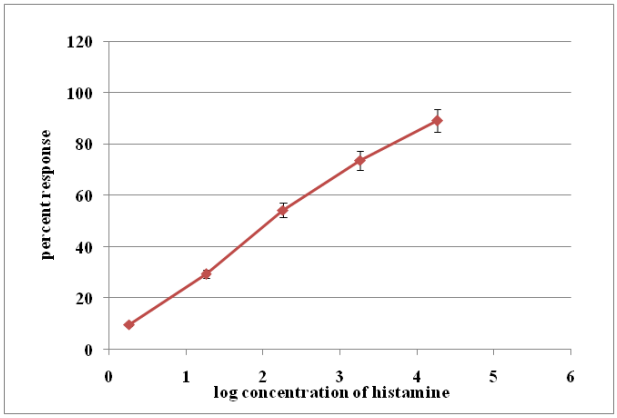

Figure 4: Response of rosiglitazone+BADGE administration

Discussion

Rosiglitazone has a significant effect on reducing contractions of tracheal smooth muscle of guinea pig by increasing concentrations of histamine and acting on the respiratory smooth muscle which shows that it can be effective in airway diseases with increased bronchial smooth muscle tone such as asthma. It was supported by a study in which rosiglitazone was observed to produce improvements in lung function as compared with inhaled beclometasone dipropionate. Bronchodilation follows administration of rosiglitazone and ciglitazone on mouse tracheal muscles.[13] The respiratory resistance in response to methacholine (MCh) is also reduced in the presence of rosiglitazone.[14]

We also explored some of the possible mechanisms for the action of the thiazolidinediones on the respiratory smooth muscle, using rosiglitazone as the investigatory tool. Among the relaxant effects of the thiazolidinediones on the isolated tracheal muscle, the first to be considered is PPAR-γ activation. Thiazolidinediones are PPAR-γ agonists and the expression of PPAR-γ increases in the airway diseases.[15] In the airway epithelium, bronchial submucosa and smooth muscle of airway tissue obtained from patients with asthma, enhanced PPAR-γ expression has been detected. [16]

Chronic airway diseases show a good therapeutic response towards agonists of PPAR owing to minimization of airway constriction and re-modeling.[17] Studies have shown PPAR agonists induced restoration of the relaxant activity produced by salbutamol. The agonists also decrease the hyper-responsiveness induced by administration of carbachol.[18] The fact that the receptor agonists other than thizolidinediones e.g. GW1929 experimentally

produce a response similar to rosiglitaone, solidifies the involvement of the PPAR. In addition, the effects are produced via the action on the gamma type of PPAR. This involvement is evident as the agonists of alpha type of PPAR e.g. fenofibrate are unable to restore the response of the respiratory muscles to salbutamol. This evidence offers a scientific basis for the use of the agonists of PPAR gamma for the respiratory conditions.

BADGE was used which is a reversible antagonist of PPAR-γ[19] and is used to study the role of PPAR-γ in the action of rosiglitazone on the tracheal muscle. The tracheal muscle was pretreated with fixed concentration of BADGE (100μM) which is also studied alone to determine its intrinsic effect as well as with rosiglitazone (100μM) for fifteen minutes. The effects of histamine were then studied on this tissue model. When the concentration response curve of BADGE and rosiglitazone pretreated group was compared with that of only rosiglitazone pretreated group, the mean values of responses as well as the mean percent responses were also found statistically non-significant ($P > 0.05$). The mean percent deviation was 2.78 percent. BADGE alone did not have any effect on the histamine concentration response curve. These observations show that rosiglitazone has an action independent of the PPAR-γ activation when acting on the tracheal muscle.

Conclusion

Rosiglitazone produced a significant decrease in the histamine induced contractions. The effect of rosiglitazone was absent in the presense of the PPAR gamma antagonist BADGE. The results solidify the hypothesis that thiazolidinediones produce the relaxation of the smooth muscles via activation of PPAR gamma

Conflict of Interest: Authors declare no conflict of interest.

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A. Conception/Study/Designing/Planning
B. Active Participation in Active Methodology
C. Interpretation/ Analysis and Discussion